ORIGINAL ARTICLE

Maternal Gestation Induced-stress Alters Reproductive Outcome in Adult Female Rats, Offspring Survival Rate and Sex–ratio

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ABSTRACT

Introduction: Investigations relating to the effect of stress on reproductive outcome, offspring survival rate and chances of still births are currently attracting global concern. The present study evaluated the effect of maternal gestation induced-stress in the altered reproductive outcome of adult female Wistar rats, offspring sex – ratio and survival following exposure to different stress models. **Methods:** The study protocol involved two parts. Seventy-eight adult healthy female Wistar rats aged between 12 – 14 weeks and weighing between 150-180g were procured and utilized for part 1 study. The rats were exposed to three stressors; restraint, mirror and intruder, respectively, for three hours per day for three weeks. For part 2 experiment twenty-four female offspring rats from the part 1 study were used. **Results:** Exposure of rats to the varying stressors increased gestate on length, decreased mean pup weights and litter size at birth especially when the rats were stressed by exposure to restraint or intruder stressor. The effect of stress on gestation period, pup weights and litter size were largely variable and dependent on the nature of stressor applied. There was significant ($p<0.05$) reduction in the survival rates of offspring of rats exposed to the different nature of stressors especially when stressed with restraint or intruder stressors. The stressors' impact was greatest when the rats were exposed to the varying stressors up to the end of the 2nd trimester and beyond. Furthermore, the male sex ratio of offspring of stressed rats was significantly ($p<0.05$) reduced than the females. **Conclusion:** Stressful maternal condition tends to compromise the fetal outcome, sex ratio specificity and the survival rate of offspring.

Keywords: Maternal stress, Gestation length, Pup weight, Litter size, Wistar rat

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INTRODUCTION

Reproduction in mammals is a complex phenomenon that is precisely regulated by hormonal, biochemical and neural processes by coordinating the central nervous system and the peripheral reproductive organs to achieve successful procreations (1). The hypothalamus's negative feedback mechanisms tightly regulate the reproductive hormonal axis; that is, the hypothalamo-pituitary-gonadal (HPG) axis is vital in the maintenance of set homeostatic control required for reproduction (2). The relationship between stress and reproduction, especially in females, is becoming a global health problem when considering population studies (3). Previous research has indicated that there are established relationships between reproduction and stressful conditions (4).

Stress experimental animals have been proven to interfere with reproductive outcome and offspring developments (5). In humans, exposure of females to maternal stress are more prone to serious risk for spontaneous abortion, obstetric complications, premature deliveries, infant mortality, birth weights reduction, abnormalities relating to infant growths and sex ratio irregularities (6); in addition to long term emotional upsets (7) and cognitive problems (8). Epidemiological proofs have demonstrated that stress has a profound negative impact on health, alongside, physiological worries related to stress during pregnancy are risk factors linked to unfavourable complications of pregnancy and reproductive outcome, such as; premature delivery, the low weight of infant birth, variations in sex ratios, still births and reduced survival chances of offspring. Animal studies relating to the effect of maternal gestation stress on reproductive outcome have focused on its effect before delivery, with few investigating the impact of pre-gestational maternal

induced-stress on delivery and growth of the offspring (9).

Offspring survival and sex ratio evaluations are critical indicators to consider in maternal gestation stress both in humans and laboratory animals. Previous studies reported that the offspring of women exposed to unpreventable stressors like; natural disasters, antagonistic life occasions or social pressures during the gestational period have a greater danger of psychopathology effects than offspring of those who were not exposed to similar stressors (10, 11). Examples of such stressors-like natural alterations are; affective disorders (12); attention deficits (13, 14); generalized anxiety states (15); depression (16) and learning deficits (17). Although, it has been established that several causes of infertility are common among women that may not be related to stress; examples include; damaged or blocked fallopian tube which often occurs often due to sexually transmitted infections (STI), endometriosis and development of pelvic adhesions, ovulation disorders and hormonal abnormalities (18).

The most delicate pointer to the impact of maternal induced-stress on the reproductive system of females and their offspring included; impaired follicular development and unsettling influence in the regular menstrual or estrous cycle or cyclicity and ovulation (19). Minor disturbances in the timing, amplitude and/or frequency of reproductive hormone secretions equally alters (generally prolonging) the estrous cycle (18). A previous study reported that mice were more sensitive than rats in their reproductive response when exposed to different stressors. However, there are differences between strains (19). Based on the above assertion, we hypothesize that rats exposed to maternal gestation stress of different stress model, demonstrates perturbed gestation periods, reduced litter size and pup weights while reducing chances of offspring survival after 4 – 8 weeks of delivery. Furthermore, we hypothesize that sex-ratio specific outcome of pups (male: female) after weaning period may indirectly influence the number of male offspring delivered by stressed Wistar rats. Therefore, this study's objective is to justify that exposure to varying kinds of stressors during gestation period alters the reproductive outcome and has maternal consequences in stress-induced female Wistar rats and its offspring.

MATERIALS AND METHODS

Experimental Design

The experimental animals were randomly distributed into different groups of six rats each (n = 6). Prior to this study a pilot study was carried out to ascertain the animals to be used for the study as reported by a study of Nwogueze et al. (20). The part 1 investigated the effect of different stress exposure during different trimesters of pregnancy on fetal outcome of female Wistar rats as shown in Table 1. The rats used for this experiment were exposed to the respective stressors at the rate of 3 hours per day for 3 weeks. The offspring from the different Wistar rats were observed for sex ratio, stillbirth and/or the survival rate of the animals as described in Table I.

Table I: Experiment on Effect of Stress on Pregnancy Outcome, Survival Rate and Sex Ratio of Offspring of Stressed Rats

Grouping	**Part 1 Experiment**	
Group 1	Control pregnant Wistar rats (Not stressed)	6 rats
Group 2	Female Wistar rats exposed to different (3) stressors before mating at 3hours per day for 3weeks	6 rats x 3 stressors = 18
Group 3[a]	Rats exposed stressors at 3hours per day for 3weeks, terminating at the end of 1st trimester of pregnancy.	6 rats x 3 stressors = 18
Group 4[b]	Rats exposed to stressors at 3hours per day for 3weeks, terminating at the end of 2nd trimester of pregnancy.	6 rats x 3 stressors = 18
Group 5*	Rats exposed to stressors at 3hours per day for 3weeks, terminated by Delivery (3rd trimester of pregnancy).	6 rats x 3 stressors = 18
Grouping	**Part 2 Experiment**	
Group A	Offspring of Wistar rats exposed to Stress	
Group B	Offspring[cd] of Wistar rats exposed to Restraint stressor of 3hours per day for 3weeks	6 rats
Group C	Offspring[cd] of Wistar rats exposed to Mirror stressor of 3hours per day for 3weeks	6 rats
Group D*	Offspring[cd] of Wistar rats exposed to Intruder stressor of 3hours per day for 3weeks	6 rats

**Exposure to stressor commenced the same day with mating.*
[a]Exposure to stressor commenced 2weeks before mating.
[b]Exposure to stressor commenced 1week before mating.
[c] Survival after 2weeks of delivery
[d] Sex ratio was determined at 8 weeks

Animal Procurement and Animal Maintenance

In part 1 experiment, 78 adult healthy female Wistar rats aged between 12 – 14 weeks and weighing between 150-180 g were procured and utilized. The animals were purchased from Emma Maria Diagnostic and Research Laboratory, Abraka, Delta State, and transported in plastic cages to the animal house of the Department of Human Physiology, Delta State University, Abraka. Prior to the study, the rats were allowed to acclimatize for a period of 14days and were fed with standard rat chaws and normal tap water ad libitum. Part 1 experiments covered a period of 5 weeks. In part 2 experiment, 24 female rats that were offspring of the stressed parent rats of part 1 experiment were utilized. Part 2 experiments covered a period of 8 weeks. In all, the animals recruited in this study were housed in wooden cages under normal room temperature and humidity, well ventilated, hygienic conditions and under normal photoperiods of about 12 hours light and 12 hours dark cycles.

Location / Duration of the Study

This study was carried out at the Department of Physiology in the College of Health Science, Faculty of Basic Medical Sciences of Delta State University, Abraka which is located in Ethiope East Local Government Area of Delta State, Nigeria. The whole experiment covered a

period of 13 weeks.

Stress Induction Protocol

The female Wistar rats were exposed to three different stressors namely; restraint, chamber test, mirror chamber test and resident intruder paradigm test (Aggressive Cat) as described in a previous study (20). The stressors were adopted to induce maternal stress at the rate of 3 hours per day depending on the period of pregnancy, that is, 1st, 2nd and 3rd trimester respectively. The stress-induced in the case of restraint stressor was considered to be essentially physical stress with minimal psychological component (21); while, the stress induced by mirror stressor was considered psychological stress (22), and the stress-induced by intruder paradigm stressor was to a large extent essentially psychological stress with a minimal physical component (23).

Ethical Considerations

Prior to the study, ethical approval was obtained from the "Research and Bio-ethics Committee of the Faculty of Basic Medical Sciences (RECFBMC)", Delta State University, Abraka, before the study's commencement. The study adopted the recommended experimental protocol and procedures for animal handling following the committee's guidelines (Permit Number: REC/FBMC/DELSU/19/58).

Mating Arrangement

The study adopted a mating ratio of one sexually-active male rat to one female rat (1:1) and each pair was maintained in a separate breeding cage. The male rats were selected randomly from a pool for mating with the female rats. The mating rats remained together in the breeding cage for not more than 3 days, until evidence of pregnancy was established. The males used for mating were never exposed to stressors.

Confirmation of Mating and Pregnancy

Each rat was identified with an indelible marker of a different colour. To confirm a successful mating, the vaginal of each of the mating female Wistar rats were examined for the presence of a copulatory plug (protein coagulates), following which a vaginal smear of each of the mated female rat was collected by carefully inserting a sterile cotton-bud tipped swab moistened with normal saline into the vaginal cavity of the rats. The swab was gently applied against the wall of the vaginal before being withdrawn. The moist swab was thereafter rolled over a clean glass slide to observe the presence of spermatozoa, such presence of which was considered the first day of gestation. This procedure was conducted twice daily (7:00 am and 7:00 pm). Having established mating, by extension pregnancy, the rats thereafter were separated, and the female Wistar rats returned to their initial grouping arrangement. Pregnancy was further confirmed by palpitation of the female rats' lower abdomen for evidence of fetuses on the 5th day after their return to the respective experimental cages.

Reproductive Outcome Parameters Determine

Gestational Length

The gestational length was determined based on pregnancy duration, which commenced from the day spermatozoa was identified in the vaginal smear of the female Wistar rats used in a specific experiment to the day of delivery. This was adopted since it will be challenging to determine the actual day conception took place.

Mean Pup Weight

After delivery, the pups available in each litter of the pregnant Wistar rats were gently dried up using filter papers and weighed using a digital electronic weighing balance. The weights of the pups per litter were added up, and the summed weight was divided by the number of pups per litter to obtain the mean pup weight.

Litter Size

On delivery, the pups were counted and then recorded as the litter size. The litter size of all the rats in a group was added up and divided by the number of rats in the group to have the mean litter size.

Stillbirth

After parturition, the numbers of pups were carefully observed and the number of pups that were delivered dead was recorded as the total stillbirths.

Percentage Survival

The survival rate was determined by the use of the formula below:

$$\text{Survival rate} = \frac{\text{Litter size} - \text{Total number of pups dead}}{\text{Litter size}} \times 100$$

Determination of Sex ratio

At 8week after gestation following fetal reproductive development of the offspring, the sex ratio variations were carefully observed for male and female sexes, after that, they were separated into weaning cages.

Statistical analysis

Data obtained were analyzed and expressed as Mean ± Standard Error of Mean (SEM) and means comparison across groups were determined by one way ANOVA using SPSS version 22 (IBM, Chicago USA) with p-value <0.05 been considered as statistically significant. Fisher's Least Square Difference (LSD) was used for post-hoc analysis.

RESULTS

Fig. 1 illustrates the effect of stress on the gestational length of female Wistar rats after exposure to stressors of different nature. For some of the rats stressing terminated just before mating, while for other rats, depending on the experimental protocol concerned, stressing terminated at

Figure 1: Effect of Stress on Gestational Length of Female Wistar Rats after Exposure to Different Stressors for 3 Weeks at the Rate of 3 Hours Per Day (n=6). *indicates a significant difference ($p<0.05$) when compared to control

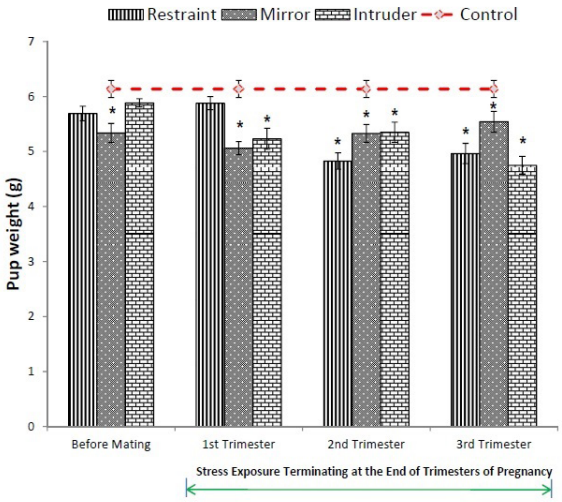

Figure 2: Effect of Stress on Birth Weight of Pups of Female Wistar Rats after Exposure to Different Stressors for 3 Weeks at the Rate of 3 Hours Per Day (n=6). *indicates a significant difference ($p<0.05$) when compared to control

the end of 1st, 2nd or 3rd trimester. The results revealed that rats exposed to restraint stressor had significantly ($p<0.05$) longer gestational length than the control value (19 days), and this is irrespective of whether the rats were stressed before mating or that stressing terminated at the end of 1st, 2nd or 3rd trimester. Also, exposure to intruder stressor extended significantly ($p<0.05$) the gestational length beyond the control length (19 days) for rats stressed before the mating and for those stressed up to the end of 1st or 2nd trimester. For rats stressed by mirror stressor, though, the gestational length increased beyond control length (19days), the increase was significant ($p<0.05$) only with respect to stressing up to the end of 2nd trimester.

Fig. 2 shows the effect of stress on pups' birth weight after exposure to different stressors. Some of the rats were stressed before mating, but for some others stressing commenced before mating and terminated at the end of the 1st or 2nd trimester. There were still some of the rats in which stressing commenced at the same time as mating and terminated by delivery. Results from this experiment showed that irrespective of the nature of stressor used in inducing stress in the rats, the birth weight of the pups remained decreased, especially when the rats were exposed to the stressors up to the end of 2nd or 3rd trimesters of pregnancy in comparison to the control pup weight (6.14g). With respect to exposure to mirror stressor, the birth weight of the pups remained significantly ($p<0.05$) decreased, when compared to the control birth weight of the pups (6.14 g). This is irrespective of the stage of termination of exposure to the stressor. In the case of intruder stressor, a significant ($p<0.05$) decrease in pup weight started when stress application terminated at the end of 1st trimester of pregnancy when compared to the control pup weight (6.14 g). But when the rats were exposed to restraint stressor, a significant decrease ($p<0.05$) in birth weight of the pups was observed when exposure to the stressful condition got up to the end of 2nd trimester of pregnancy and beyond.

Fig. 3 illustrates the effect of stress on litter size after exposure to different stressors up to the end of the 1st, 2nd or 3rd trimester and before some of the rats was mated. The average litter size of rats exposed to restraint stressor before they were mated was significantly ($p<0.05$) decreased when compared to the average litter size of the control rats (8.67). Exposing rats to restraint stressor up to the end of 1st, 2nd or 3rd trimester of pregnancy did not significantly alter the rats' litter size. As for the exposure to mirror stressor, stressing the rats before mating them did not alter the rats' litter size. But stressing the rats up to the end of 1st trimester of pregnancy significantly ($p<0.05$) decreased the litter size of the rats. The litter size when the rats were stressed up to the end of 2nd trimester of pregnancy was though decreased, but not significantly different from the litter size of the control rats. However, stressing the rats up to the end of pregnancy significantly ($p<0.05$) decreased the litter size. Stressing the female rats by intruder stressor either before mating or up to the end of the 1st trimester did not significantly alter the rats' litter size, though, the average litter size decreased compared to the average control litter size (8.67). Nevertheless, when the rats were stressed up to the end of the 2nd trimester, litter size significantly ($p<0.05$) decreased in comparison to the litter size of the control rats (8.67).

Fig. 4 comparatively shows the mean distribution of still births by pregnant rats stressed by exposure to restraint, mirror or intruder stressor up to the end of the 1st, 2nd or 3rd trimester. The list number of still birth was observed among rats exposed to restraint stressor while the highest number was among rats stressed by exposure to mirror stressor (as shown in Figure 4A). Still birth was prominently observed when rats were exposed to the

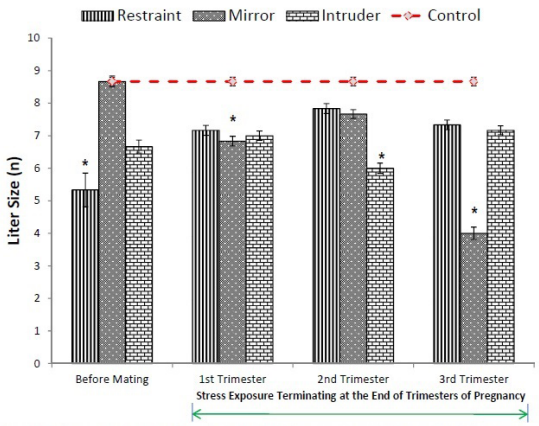

Figure 3: Effect of Stress on Litter Size of Female Wistar Rats after Exposure to Different Stressors for 3 Weeks at the Rate of 3 Hours Per Day (n=6). *indicates a significant difference ($p<0.05$) when compared to control

Figure 5: Survival Rate of Offspring of Wistar Rats Stressed by Exposure to Different Stressors for 3 Weeks at the Rate of 3 Hours Per Day (n=6). *indicates a significant difference ($p<0.05$) when compared to control

end of 3rd trimester of pregnancy irrespective of the stressor applied (Figure 4B). It is important to note that the number of still birth were not more than 2 pups per litter irrespective of the stressor applied.

Fig. 5 shows the percentage of survival of offspring of parents stressed by exposure to stressors of different nature after 14days of delivery. The survival rate of the offspring in all the situations studied decreased compared to that of the control. However, the decrease in survival rate became significant ($p<0.05$) for offspring of the parent female rats stressed by exposure to restraint and intruder stressors when the stressors were applied up to the end of the 2nd and 3rd trimester of pregnancy. With respect to exposure to mirror stressor, the survival rate of offspring significantly ($p<0.05$) decreased only when stress was applied up to the end of pregnancy (i.e delivery time).

Fig. 6 shows the effect of different stressors on the specific sex ratio variation of offspring at the rate of 3 hours per day for 3 weeks up to the end of 1st, 2nd and 3rd trimester and when some of the rats were exposed to stress before mating. Results obtained revealed that stressing the rats with restraint, mirror or intruder stressor significantly ($p<0.05$) reduced the number of male offspring than the female offspring compared to the control rats. Stressing the rats by intruder stressor before mating or up to the end of 2nd trimester or beyond, proven to be more deleterious and significantly ($p<0.05$) reducing male pups' sex ratio when compared to the male offspring of rats exposed to mirror or restraint stressor. The sex ratio of females was unaffected by exposure to different stressors. However, stressing the rats with mirror or intruder stressor up to the 3rd trimester of pregnancy significantly ($p<0.05$) reduced the female offspring compared to the control rats.

DISCUSSION

In the present study, female Wistar rats were exposed to restraint stressor, mirror chamber stressor and resident intruder stressor and it was observed that the responses of the rats to the different stressors varied with regards

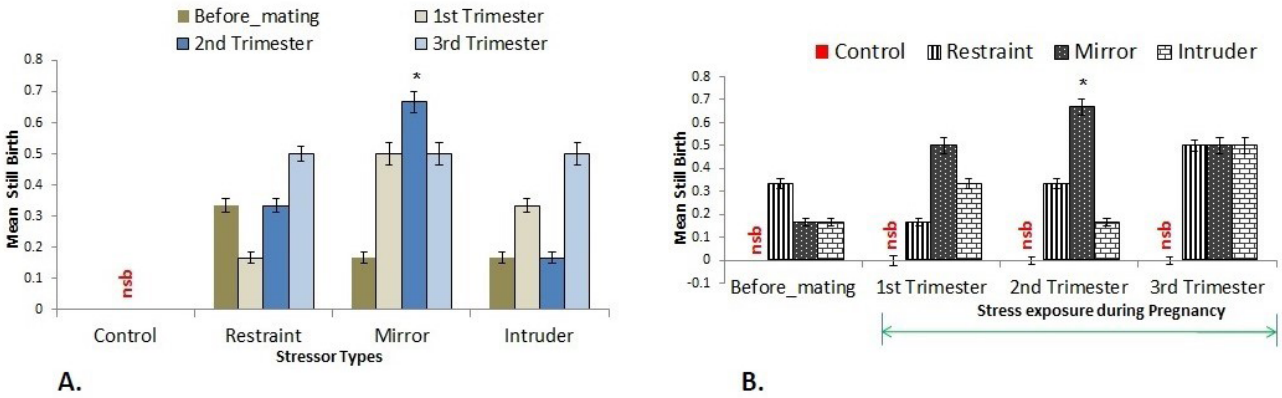

Figure 4: Still Births by Rats Stressed by Exposure to Different Stressors for 3 Weeks at the Rate of 3 Hours Per Day (n=6). *indicates a significant difference ($p<0.05$) when compared to control. nsb indicates No Still Birth.

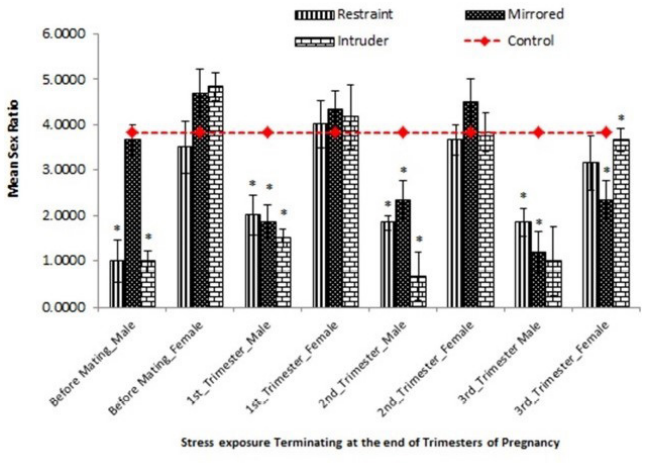

Figure 6: Effect of Stress on Sex Ratio of Offspring of Female Wistar Rats after Exposure to Different Stressors for 3 Weeks at the Rate of 3 Hours Per Day (n=6). *indicates a significant difference ($p<0.05$) when compared to control

to the reproductive outcome, suggesting that responses of rats to exposure to stressors depends, at least to some degree on the extent of stress-induced in the respective rats. This study revealed that irrespective of the stressor, the gestation lengths were more affected when the rats were exposed to the stressor up to about day 14 of the gestation period. This explains why a previous study maintained that the stage of gestation during which stress was applied is crucial in establishing their effects on reproductive outcome and subsequently post-natal development (24). Previous studies have established that the HPA-axis exhibits a reduced response to a wide range of physical and psychological stressors during gestation (25). Probably, in the absence of this modified role of HPA – axis, the consequence of stress on pregnancy outcome would be overwhelming.

Stress had prominent effect on gestation period and mean pup weight at birth of the Wistar rats, especially when the rats were stressed by exposure to restraint and intruder stressors. The gestation period was increased and the mean pup weight at birth was decreased. It is possible that changes in maternal milieu induced by stress as suggested in previous studies that during gestation influences the pregnancy outcomes of the fetus (26). This study's finding concerning gestation length is consistent with that of previous study who found that there is significantly longer gestational length in rats subjected to physical restraint stressor in early pregnancy (27). The authors remarked that restraint stressor during maternal gestation alters normal fetal development (27). From the present study the impact on gestation length was least when the stress was applied in the third trimester of pregnancy. This conclusion is based on the finding that with respect to restraint and intruder stressors, the gestation length was not significantly altered when stress was applied up to the third trimester. In contrast to this study's finding concerning gestation length, exposure to stress during gestation did not have a significant effect on gestation length (28). It is possible that the nature of the stressor used and the duration of exposure of the rats to the stressor may account for the different observation made.

Findings from this study show a significant decrease in pup weights following exposure to different nature of stressors. This is supported by previous reports that showed that exposure of rats to prenatal stress has implications for pregnancy outcome, thus causing a reduction in birth weights of the pups (29, 30). It has been established that this could be as a result of the cascade of neurohormonal events which triggers HPA axis hyperactivity following stress response (31). The pub weights were affected more in the mirror and intruder groups. Reduction in birth weights is an indicator of altered fetal developments which has a link to corticotrophin-releasing hormone (CRH) secretion from the hypothalamus and adrenocorticotropic hormone (ACTH) from the pituitary leading to an increase in cortisol level due to stress (32).

The results obtained from the present investigation revealed a reduction in litter size following exposure of rats to different stressors across different trimesters. In all cases, irrespective of the nature of stressor involved in stressing the rats, liter size was decreased. This finding is in line with earlier study that reported that rats exposed to gestational restraint stress had significantly smaller litters than the control dams (33). Prenatal stress has been associated with adverse pregnancy outcomes, such as reduced litter size and survival rates (25). The possible mechanism of litter size reduction in gestational stress as reported in our study may involve oxidative-stress-mediated alteration in ovarian and placental functions vital for embryonic growth development, as explained in (34).

Stillbirths were observed following exposure to maternal stress in stressors of different nature at the varying period of pregnancies compared to the unstressed control rats had no record of stillbirths and neonatal mortalities. As reported in previous research, stillbirths were considered one of the leading causes of neonatal mortality globally and are often linked to several mechanisms that implicate stress-induced maternal conditions (35). In the current study, the observed stillbirths were highest in female Wistar rats exposed to mirror stressors especially at the 3rd trimester of pregnancy while the stillbirths were least following exposure to restraint stressor. Maternal stress exposure during the gestation has possible antagonistic impacts on the developing fetus, resulting in the onset of varying pathological neonatal conditions (36). In particular, the offspring of stressed female Wistar rats exposed to physical restraint stressor during the late and full-term gestational period indicated progressively harmful impacts, such as physical impairment, neonatal mortality, stillbirths, and low birth weight, preterm births and delayed eyelid opening (35, 36).

Exposure of Wistar rats to stress caused significantly decreased in the pup's survival rate, of their pups especially when restraint or intruder stressor was used to stress the rats. The stressors' impact was greatest when the rats were exposed to the stressors up to the end of the 2nd trimester and beyond (i.e day 14 and above of the gestation). The restraint and intruder stressed rats were affected more. These findings is supported by the research outcome of previous clinical study who reported that violence against pregnant women or pregnant women experiencing stress had increased risk of infant and child mortalities; thus, exposure of rats to prenatal maternal stress have greater chances of offspring defects and still births as well as the risk of neonatal mortality (37). This study establishes that the offspring of rats exposed to restraint or intruder stressor up to the second and third trimesters of pregnancy have fewer chances of survival than those exposed to mirror stressor. This further confirms previous research findings that restraint stress during pregnancy causes still births and altered maternal health in rats (35).

Maternal induced-stress during gestational periods of pregnancy has been shown to have profound effects on sex-specific variation in offspring outcome. In this study, exposure to the stress of different nature reduced male to female offspring in the different trimesters of pregnancy irrespective of the stressor involved. The mechanism responsible for such observed stress-induced alterations in the maternal physiology remains unclear. The previous study has established that such related differences observed in sex ratio in response to fetuses to the adverse maternal environments suggest that the male developing fetuses are more likely to be susceptible to changes in the maternal environment than the female fetuses (38). Furthermore, other studies, supported this view by adding that the male fetal reproductive axis is more sensitive to environmental change than the female reproductive axis (39). Although, other studies attributed that reasons for such sex variations remains unknown (40). However, an earlier experimental study maintained that variable impact of stress-induced alterations often explain sex differences in response to maternal gestation induced-stress in the fetal reproductive HPA axis on the male and female fetal hypothalamic–pituitary–gonadal (HPG) axis (42).

CONCLUSION

We conclude that maternal gestation induced-stress alters reproductive outcome due to exposure to different stressors, and such effect may be of different quality in the Wistar rats. Thus, this may explain the female Wistar rats' variable responses to varying stress models in different trimesters of pregnancy. In all, continued exposure of the rats to the stressful condition tends to compromise the fetal outcome and the survival rate of offspring while causing observable stillbirths. Gestational length increased when rats were stressed early (within the 1st 2 weeks) and not last week. Stressing pregnant rats till the end of the 2nd trimester and beyond significantly reduced the offspring's survival rate more than when stressed earlier. The study further established that exposure to intruder or stressor is more stressful to the rats than the mirror or the restraint stressor. Whereas, exposure to maternal gestation induced stress may enhance the development of reduced chances for male offspring. Therefore, it becomes necessary to recommend that pregnant women be taken out of an atmosphere of stress.

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